CD20, CD5, CD23, Ki-67, Bcl-2, CD10, CD45, CD30. AE1/3, S100, CD34, Desmin, CK5/6

A pragmatic approach to Lymphoma diagnosis and lymph node evaluation in a resource-poor setting such as Nigeria.

Chapter 1. Introduction

The histopathologic diagnosis of lymphoma and evaluation of lymph nodes has become increasingly complicated over the past few decades, so much so that in the developed world where all required facilities are available, this task is increasingly left in the hands of the few pathologists who are prepared to expend the energy required to keep up with this complex task. A few decades ago, the classification of lymphoma was dependent almost entirely on assessment of cellular morphology and tissue architecture in the context of clinical presentations (Kiel, Luke-Collins, Working formulation). These days it is no longer sufficient to diagnose lymphomas as broad groups, rather each entity is considered a unique disease with often distinct clinical management options and prognosis. Currently classification of lymphomas is so heavily dependent on the immunohistochemical characteristics (and increasingly on molecular genetic markers) that one can hardly make any useful diagnosis of a lymphoma without immunohistochemistry.

In developing parts of the world such as Nigeria, facilities for immunohistochemistry are either not available routinely or are rudimentary with very limited number of antibodies. In most instances the only tests being performed for research or under the auspices of drug companies interested in marketing their products are hormonal assessments for breast cancer. Consequently the majority of practicing pathologists and trainees in Nigeria have limited exposure to the use of immunohistochemistry in routine histopathology. Most of their knowledge in this area may be theoretical from literature or attendance at conferences/training events. As pathologists become more conscious of the need for it and clinicians on behalf of patients demand more detailed diagnosis on lymph node samples, the way out will usually be to send the readily portable paraffin wax blocks of tissue samples overseas for proper assessment with immunohistochemistry. Indeed as pathologists become more conscious of how limited their assessment of lymph nodes is without the aid of immunohistochemistry there will be the tendency to send virtually every sample that could possibly be a lymphoma away. This trend if unchecked can only eventually result in a very poorly developed skill base for lymph node assessment within the country which will remain as a serious problem when immunohistochemistry facilities become universally available. There are two aspects to the introduction of immunohistochemistry into routine histopathology practice in Nigeria. The technical aspect includes everything involved in eventually producing the immunohistochemistry stained slide. The interpretational aspect which is the domain of the pathologist involves the skill required to appropriately request and then interpret the stained sections. While the technical aspect can readily be overcome with financial investment in the required infrastructure and relatively short-term (albeit continous) training of laboratory scientists, the interpretational aspect will require long term training/experience and use on the job for pathologists.

This monograph is therefore being written with the aim of finding a practical way for practicing pathologists in Nigeria to be gainfully involved in diagnosis of lymph node

diseases including lymphomas within the limits of currently available resources. By attention to detail and lateral thinking, a very limited panel of 8 carefully selected antibodies (CD10, CD20, CD5, CD23, Ki-67, Bcl-2, CD30 and CD45) together with 5 other antibodies for more general histopathology use (AE1/3, S100, CD34, Desmin, CK5/6) can be employed to diagnose a majority of the lymphomas that are likely to be encountered, exclude lymphoma/diagnose non-lymphomatous diseases of the lymph node in some cases and thereby appropriately refer overseas only those cases that really require a more extensive array of antibodies or molecular genetic analysis. This approach will ensure that there is a skill base available in the country to grow with the anticipated increasing availability of material resources in terms of antibodies and molecular techniques that will be expected with economic advancement in the country.

This write-up will deliberately be sketchy and aim to avoid unnecessary repetition of details that are readily available in well known books and publications, but will focus on salient points that will aid this proposed pragmatic approach. At every step, the limitations will be mentioned so that the reader is aware of when to seek external help. The idea is to deploy only a few antibodies which will be used frequently enough in a non-research setting to be economically viable/sustainable in the current economic climate.

Chapter 2. Review of lymph node anatomy with the selected antibodies

The anatomy of the lymph node as described here presumes that an intact node has been sectioned through the hilum longitudinally. It is important to keep this in mind because that will not be the case for most lymph nodes that one will come across. Besides, incisional and needle core biopsies of lymph nodes are increasingly common. Hence one needs to be imaginative when examining lymph nodes since what may appear to be a deviation from the normal described here may only be a result of the way in which the sample has been taken (e.g. direction of travel of the biopsy needle) or cut during laboratory handling/processing.

The lymph node is enclosed by a fibrous capsule beneath which is the sub-capsular sinus that receives afferent lymphatics. The sub-capsular sinus continues into the medullary sinuses which drain toward the hilum of the lymph node from where efferent lymphatics originate. The main contents of the sinuses are sinus histiocytes and small numbers of circulating small lymphocytes.
The lymph node substance is divided into B and T cell zones. The B-cell zone is mainly represented by the lymphoid follicles. The skeleton of the lymphoid follicle is formed by a meshwork of the cytoplasmic processes of the follicular dendritic cells which are antigen processing/presenting cells of the macrophage histiocytic cell line. This meshwork which can be imagined as resembling a native sponge is the defining structure of the lymphoid follicle. That is if there is no FDC meshwork then whatever one might have seen on the H/E slide that looked like a follicle is not. Conversely if immunohistochemistry with the specific markers defines an FDC meshwork when a follicle was not apparent on the H/E, then there is indeed a follicle! There are a number of antibodies to outline the FDC meshwork. CD21 and CD23 are commonly used together because they stain somewhat differently and there are situation where one does outline FDC meshwork that has not been picked up by another. We have selected CD23 into our panel because it has other uses such as in the diagnosis of CLL/SLL. Even when CD23 fails to stain FDC meshworks, the presence of follicles may be reliable inferred by other markers in our panel in some situations as will be outlined later. However it is important to keep in mind that having only CD23 in our limited panel does mean that there is some significant limitation in confirming the presence of true lymphoid follicles. Also note that CD23 stains a small population of B-cells and this must not be confused with the actual processes of the follicular dendritic cells which form the meshwork. In a normal or mildly reactive lymph node sectioned as we have described, lymphoid follicles are predominantly peripherally located in the cortex

The vast majority of the cells in the lymphoid follicle are B-cells. There is a host of antibodies that identify a broad range of B-cells commonly referred to as pan B-cell markers. Two common ones often used together because they cover a broad spectrum of B-cells are CD20 and CD79a. Either or both antigens are expressed at every stage of maturation of B-cells from the early lymphoblast to the late plasma cells. There are of course rare occasions when a B-cell is not identified by either marker. We have chosen CD20 as the B-cell marker in our panel because it is not only a very robust and easy to use antibody but also is very widely expressed in B-cells and is the target of a very important drug in the treatment of B-cell lymphomas (Rituximab). Two type of follicles are noted – the primary and the secondary (stimulated or activated) follicles. The B-cells of the primary follicle are small mature cells which can be

regarded as being in a resting state in contact with the follicular dendritic cells. When these are stimulated by antigen that has been processed and presented by the follicular dendritic cells, there is a host of changes that result in the development of an enlarged germinal centre populated by cells that are collectively referred to as follicular centre cells. The main cells of the germinal centre are the centroblasts (which are the proliferating cells) and the centrocytes. Both of these are B-cells. A tiny population of T-cells (follicular centre helper T-cells) are also present and are characteristically positive with CD57 as well as pan T-cell markers. There is also of course the main body/nucleus of the follicular dendritic cells and the tingible body macrophages that is seen in reactive germinal centre engulfing apoptotic debris. The follicular centre (B) cells are a unique population which have a number of specific markers including CD10 and Bcl-6. Again the two markers differ somewhat in their sensitivity for these cells and it is not uncommon to find situations in which one is positive but the other is not. We have chosen CD10 for our panel because it is robust and has other uses such as in marking metastasis from classical renal cell carcinoma and marking endometrial stromal cells.

The T-cell zone is between the lymphoid follicles and includes the medullary cords (between the medullary sinuses) of the lymph node. The vast majority of cells in this zone are small mature T-cells with a tiny population of larger proliferating blasts. These are marked by pan T-cells markers such as CD3 and CD5. We have chosen CD5 because it has other uses including diagnosis of CLL/SLL and raising the possibility of mantle cell lymphoma (usually confirmed with cyclin D1). There is also the interdigitating reticulum cells which is the counterpart of the follicular dendritic cells albeit with different characteristics and markers (such as S100). And there are the ordinary histiocytes as well as occasional eosinophils and neutrophils.

Chapter 3. A simplified approach to lymph node evaluation

Virtually all lymph nodes biopsies examined by a pathologist will by definition be abnormal; otherwise it would not have been enlarged or biopsied. Abnormalities of the lymph node can affect only a single compartment when they are subtle or in early phase. It is therefore vital that a systematic examination of all compartments of the lymph node be done routinely so that important abnormalities are not missed. For instance a lymph node may have only subtle involvement by an anplastic large cell lymphoma that is limited to the sinuses and Classical Hodgkin's lymphoma may be limited to the paracortex with very little alteration to the lymph node architecture.

Common lymph node abnormalities can be broadly classified into

A) Metastatic disease
B) Specific reactive/inflammatory changes
C) Non-specific reactive/inflammatory changes
D) Lymphomas

A) Metastatic disease to the lymph node is commonly from carcinomas and melanomas and should be fairly easy to diagnose as it involves replacement of parts of the lymph node by wholly foreign and often atypical looking cells. The little important fact to keep in mind is that there is rarely a situation in which benign structures such as epithelial or naevus cells are entrapped in lymph nodes which often have other abnormalities. These rests are often very small and appear bland and should be definitely diagnosed in the correct clinical context without the necessity of resorting to extensive/expensive search for a primary tumour. The epithelial nature of a metastatic tumour can be confirmed by staining with a broad spectrum cytokeratin such as AE1/3 in our panel while melanoma would nearly always be positive with S100.

B) A typical specific reactive change in the lymph node would be granulomatous lymphadenitis. These include caseating granulomas like tuberculosis, non-caseating compact granulomas like sarcoidosis and histiocytic palisading granuloma like lymphogranuloma venerum or cat scratch disease.
While the morphologic features of such diseases may be relatively familiar, it is important that pathologists still carefully examined all compartments of a lymph node. This is because not only is it possible for dual pathology to be present in the lymph node but also some lymphomas such as Hodgkin's may have a reactive component that includes granulomata.

C) Non-specific reactive/inflammatory changes may involve a single or a combination of the lymph node compartments. Enlargement of the sinuses with increased numbers of the normal contents of histiocytes known as sinus histiocytosis is often seen in lymph nodes that are draining regions involved by tumour. Again it is vital to carefully examine such expanded sinuses to ensure that the contents are merely histiocytes and not lymphoma cells or metastatic carcinoma.

Expansion of the paracortical/T-cell zones is a reactive change often seen in viral infections or reactions to drugs. The reaction is characterised merely by variably increased numbers of cells types that are normally present with some alterations in proportions. It is importantly to examine closely to ensure that cells which are not

native to this zone are not present as this would usually indicate some other specific disease including malignant lymphomas.

Reactive changes involving the B-cell zones/lymphoid follicles are commonly seen in lymph nodes draining sites of bacterial infections/abscesses. Lymphoid follicular hyperplasia is characterised by the development of pale germinal centres/formation of secondary follicles.
Benign secondary lymphoid follicles have certain distinguishing features from malignant follicles as seen in follicular lymphoma. The follicles tend to be mostly in the cortex of the lymph node and are of varying sizes. The follicles show polarity of their mantle zone which means this zones is much thicker at one end (usually that nearer the capsule) than at the other. There is also zonation of the germinal centre which means that the proliferating centroblasts are concentrated in one half of the germinal centre (usually that away from the capsule). Finally there is an abundance of tingible body macrophages. As mentioned before benign follicle (germinal) centre cells are always negative for Bcl-2.
When there is a departure from these well-defined abnormalities, then a malignant lymphoma is a serious consideration and a pragmatic approach to assessing for this will be set out in the next section.

Chapter 4. Common lymphomas that can be diagnosed with our limited panel.

The current WHO classification of lymphoid and haematopoietic malignancies lists up to 25 distinct lymphomas. A small number of these entities make up the majority of malignant lymphomas. As is often the case in many other diseases, reliable figures/statistics from Nigeria and Black Africa are not available so we have to make assumptions using figures from developed countries.

1) Hodgkin's lymphoma is said to make up to 30% of all lymphomas
2) Follicular lymphoma makes up to 20% of all lymphomas *(and primary cutaneous follicular centre cell lymphoma make up to 60% of all cutneous B-cell lymphomas)*
3) Diffuse large B-cell lymphomas make up to 20% of all lymphomas *(and primary cutaneous diffuse large B-cell lymphomas make up to ????? of all cutaneous B-cell lymphomas)*
4) B small cell lymphocytic lymphoma/CLL makes up to 4.5% of all lymphomas and
5) Nodal Marginal zone lymphoma makes up to 1.8% of all lymphomas

Overall therefore these five distinct lymphoma types (or broad class in the case of Hodgkin's lymphoma) comprise over 75% of all lymphomas! We believe that when these specific lymphoma types present with fairly typical features they can be confidently diagnosed with due attention and care to both morphologic and clinical features with only the antibodies in our selected limited panel. This means that even if up to one-third of these lymphomas present in a very atypical manner, then up to 50% of all lymphomas that a typical surgical pathologist in Nigeria will come across can be diagnosed locally leaving the other 50% to be referred overseas.

Furthermore there are a couple more lymphomas of importance that can be diagnosed with our limited panel including

1) Burkitt's lymphoma which as sporadic type forms 1-2% of all lymphomas in Western Europe but as endemic type forms 30-50% of all childhood lymphomas in Nigeria. It is clinically vital to accurately distinguish this from other childhood lymphomas.
2) Extranodal marginal zone lymphoma/Mucosa Associated Lymphoid Tumor (MALT) which forms 7-8% of all B-cell and 50% of all gastric lymphomas

We can then see that despite the limited resources available, a practicing pathologist in a resource poor setting such as most of Nigeria can do some significant work in the area of lymphoma diagnosis and build a strong foundation for the future when hopefully more resources will be available. The key to this approach is lateral thinking, careful assessment of morphologic features against the normal on both H/E stained sections and sections stained with the limited antibody panel and most importantly knowing and accepting the limitations with the option to refer externally for further assessment when definitive diagnosis is not possibly.
We shall now proceed to set out how to diagnose each of these entities with our limited panel of antibodies with notes on potential pit-falls.

Chapter 5: A simplified approach to diagnosis of common lymphomas

The diagnosis of lymphoma becomes a serious consideration when

a) There is total or partial effacement/obliteration of the normal lymph node architecture (of B and T zones and sinusoids) by excessive numbers and abnormally located specific type of lymphocytes, or
b) The lymph node architecture is partially or totally effaced by what appears to be a reactive/inflammatory infiltrate in which careful scrutiny identifies a small scattered population of abnormal lymphoid cells. This is the usual pattern for classical Hodgkin's lymphoma, but may also be seen in entities like T-cell/Histiocyte rich diffuse large B-cell lymphoma.
c) The lymph node architecture is essentially intact but abnormal lymphoid cells that are essentially foreign to the lymphnode are identified – as may be the case sometimes with identification of Reed-sternberg cells in the interfollicular areas or anaplastic large cell lymphoma cells in the sinusoids. This emphasises the importances of careful examination of all compartments of the lymphnode.

There are three important questions to ask when the lymph node architecture is effaced by a lymphoid lesion.

1) Does the lesion have a follicular or a diffuse architecture
2) If diffuse, are the majority cells neoplastic or reactive?
3) Especially if diffuse, can the lesional cells be identified as B (CD20+ve/CD5-ve) or T (CD5+ve/CD20-ve) with the markers in our panel?
4) If diffuse and majority cells are neoplatic, is it of low or of high mitotic activity (grade) whether by count of mitotic figures or using Ki-67 index?

Lymphomas with a follicular architecture

When the lymphoid lesion appears to have a follicular architecture, it is important to confirm this by identifying FDC meshwork with CD23. On the other hand, sometimes especially in small needle core biopsies, the follicular architecture may not be apparent until sections are stained with CD23. As stated before, the fact that CD23 does not stain the FDC meshwork in every instance where they exist is a limitation that has to be accepted if we want to use a small panel. Where the existence of true follicles cannot be reliably inferred from other features (with experience), then such a case would be one that needs referral for assessment with a broader range of antibodies.

Another issue to keep in mind is to distinguish neoplastic from residual follicles. Neoplastic follicles would be integral to the lesion, multiple and closely packed, while residual follicles would be peripheral to the lesion, compressed and spaced out whilst retaining many features of benign lymphoid follicles as previously described.

The primary consideration in any lymphoid lesion with a follicular architecture is follicular lymphoma. There are however mimics some of which are uncommon but need to be excluded. The commonest mimic presenting a difficulty is a benign

follicular hyperplasia. Other uncommon mimics are when benign follicles are colonised by neoplatic cells of other low-grade lymphoma which then present with a follicular architecture such as may be seen in some cases of nodal marginal zone lymphoma or mantle cell lymphoma. Another rare mimic is nodular lymphocyte predominance Hodgkin's lymphoma.

The best approach to avoiding these mimics is to stick strictly to the features of a follicular lymphoma. A follicular lymphoma is a malignant neoplasm of follicular centre B-cells i.e. a follicular centre cell lymphoma forming follicles. There are rare cases of follicular centre cell lymphomas that do not form follicles which are referred to as diffuse type follicular centre cell lymphoma. And on occasion follicular centre cell lymphomas may be partly diffuse and partly follicular (mixed).
The important thing is to keep in mind that neoplastic follicular centre cells are distinguish from benign follicle centre cells by the fact that they have lost vital growth regulatory mechanisms. The most important of this which is diagnostically useful is the abnormality in the apoptosis mechanism manifested by the expression of the Bcl-2 protein identified in over 95% of follicular centre cell lymphomas. So whereas benign follicle centre cells are always Bcl-2 negative (not forgetting that follicle centre T-cells would express Bcl-2 as would mantle zone B-cells), neoplastic follicle centre cells are in over 95% of cases Bcl-2 positive (a different mechanism is responsible for the growth regulation abnormality in the other 5%).

In summary to diagnose follicular lymphoma, the suspect follicles must be formed by follicle centre B-cells (CD20, CD10 positive) that express Bcl-2. The major limitation here is of course that CD10 does not stain absolutely all cases of follicle centre cells and where this is the case, then a referral for assessment with a broader range of antibodies and a consideration of other possibilities is warranted.
Follicular lymphoma has three grades. Grade 1-2 inlcudes cases in which less than 15 blast cells are counted in a random high power field. In grade 3a there are more than 15 blasts/HPF but centrocytes can still be identified. In grade 3b centrocytes are not readily identified, and the follicles seem to comprise of blasts only. It is not uncommon to find that in parts of a grade 3b follicular lymphoma the FDC meshork is disrupted and the blasts are growing in sheets, indicated transformation to a diffuse large B-cell lymphoma.
It is not necessary to assess all follicles with immnunohistochemistry in order to exclude follicular lymphoma. Benign follicles seen in follicular hyperplasia of the lymph node have distinguishing characteristics which have been described earlier including, peripheral/subcapsular location, polarity of well defined mantles, zonation and tingible body macrophages. However where all the features of benignity are either not present together or unclear, it may be worthwhile confirming with the finding that the germinal centres are Bcl-2 negative. And where there is serious doubt about the benign nature of the follicles even when Bcl-2 is negative, because of almost complete absence of any of these benign H/E features then the possibility of a Bcl-2 negative follicular lymphoma must be considered. Again such cases would be ones for referral for more experienced assessment with a broader range of antibodies.

When pre-existent follicles are colonised by marginal zone lymphoma, the neoplastic cells would be CD20 positive, but CD10 negative as well as CD5 negative. When follicles are colonised by Mantle cell lymphoma, the neoplastic cells would be CD20 positive, but CD10 negative and in nearly all cases CD5 positive. Marginal zone

lymphoma as described later can be confirmed as a diagnosis of exclusion for low-grade lymphomas, but Mantle cell lymphoma diagnosis requires positive staining with Cyclin D1 (or rarely D2 and D3) which is not in our limited panel.

Finally a lymphoma in which a follicular architecture may be seen is Nodular lymphocyte predominance Hodgkin's disease. Again, though the vast majority of the cells present in the nodules which do have FDC meshworks are small B-cells (CD20+ve) they are not follicle centre cells (CD10 negative). The follicles of NLPHD are usually very large and appear to comprise a sea of small mantle cells in which sometimes, multiple small germinal centres are present. This can give the appearance of a merger of follicles which is often referred to as progressive transformation of germinal centres. At this stage there is sometimes some reactive germinal centres in the background. In more established disease these germinal centres disappear and the nodules comprise only the small B-cells with usually a smaller population of small T-cells. The diagnostic variant Reed-Sternberg cells (the popcorn cells) are mostly located at the periphery of the nodules. They unlike the majority of their counterparts in the classical Hodgkin's are CD15 negative and CD20 positive. In addition the cells are immediately surrounded by a cuff of small T-lymphocytes which will stain with CD5 but not CD20.

Diffuse lymphomas with majority reactive cells

Every lymphoma has a population of non-neoplastic/reactive cells which may be a single population of lymphocytes or a mixed population of various leucocytes including lymphocytes, histioctytes, eosinophils e.t.c. The proportion of reactive cells in a lymphoma varies greatly from barely significant in for example Burkitt's lymphoma to overwhelming in T-cell and histiocyte rich diffuse large B-cell lymphoma. It is important to keep the reactive cells in mind for a number of reasons.

a) when the reactive cell population forms the vast majority of cells present and is of a single population, it is possible for this to be mistaken for the neoplastic population and the neoplastic cells are obscured
b) the presence of a heavy reactive cell population may complicate the assessment of immunohistochemistry when as would often be the case double staining is not available.

The typical lymphoma in which the reactive cell population forms the vast majority of cells present is Hodgkin's lymphoma. Diagnosis of Hodgkin's lymphoma is dependent on identifying the classical or variant Reed-Stenberg cells in a suitable background which varies depending on the type of Hodgkin's lymphoma. It is important to be strict about the criteria because RS-like cells may be seen in inflammatory and neoplastic conditions of lymph nodes other than Hodgkin's lymphoma.

Two classes of Hodgkin's lymphoma exist

a) Nodular lymphocyte predominance Hodgkin's lymphoma
b) Classical Hodgkin's lymphoma.

Nodular lymphocyte predominance Hodgkin's lymphoma has been discussed previously under lymphomas with a follicular pattern. In this context it is essentially a lymphoma in which the reactive/background cells is majority small B-cells which

are organised as nodules/large follicles. There is also a much smaller population of reactive/background small T-cells some of which immediately surround the neoplastic RS cells in a rosette. The population/proportion of T-cells seems to increase as the disease progresses. It is important to remember that NLPHL is almost certainly an entirely different disease from classical Hodgkin's. Not only do the RS cells in NLPHL have a different immunophenotype, but also the disease has an entirely different course. It remains indolent for long periods and progress by transformation into a T-cell/histiocyte rich diffuse large B-cell lymphoma.

Classical Hodgkin's lymphoma has four types

1) Nodular sclerosis
2) Mixed cellularity
3) Lymphocyte rich
4) Lymphocyte depleted

The RS cells in classical Hodgkin's lymphoma will have a typical immunophenotype using our panel of CD30 positive/CD45 negative and usually CD20 negative. In contrast in NLPHL they would be CD20 positive, CD45 positive (and usually CD30 negative) with a rosette of CD5 positive small T-cells. The majority of background cells would be small B-cells (CD20 positive) which are organised as nodules/large follicles.

Hence in cases where the morphology of the RS/RS-like cell is clear and the background is appropriate, a safe diagnosis of classical Hodgkin's lymphoma can be made if the RS cells are CD30positive/CD45 negative.

The background population in Nodular sclerosis and mixed cellularity Hodgkin's is similar comprising a variable mixture of small lymphocytes, histiocytes, eosinophils, neutrophils and fibroblasts. Nodular sclerosis Hodgkin's differs from mixed cellularity in having a thickening of the fibrous capsule of the lymph node and a nodular architecture due to intersecting fibrous bands that extend into the substance of the lymph node from the thickened fibrous capsule.

Lymphocyte rich Hodgkin's lymphoma would have a background of small mostly T lymphoctyes that often obscure the RS cells making them difficult to identify. In lymphocyte depleted Hodgkin's there is extensive sclerosis of the lymph node with relatively few lymphocytes, and the RS cells are more easily identified.

The other lymphoma of importance in which the reactive cells form a vast majority of the cells present is the T-cell/Histiocyte rich diffuse large B-cell lymphoma. As is obvious from the name the background cells are small T lymphocytes and histiocytes in varying proportions. The neoplastic cells are very few, comprising B lymphoid blasts (CD20 and CD45 positive). There is some potential for confusion in the mind of the reader between this entity and Nodular Lymphocyte predominance Hodgkin's especially when it is said that NLPHL has a diffuse type without nodules/large follicles. The important distinction is that in TCHR DLBCL, the majority reactive cells are small T lymphocytes unlike in NLPHL where they are small B lymphocytes (which in the vast majority of case do form the nodules/large follicles).

A type of lymphoma that may fit into this group is angioimmunoblastic T-cell lymphoma and other similar types of T-cell lymphoma. They are relatively rare and though a definite diagnosis cannot be made, ought to be picked out for onward referral because of the fact that while being obviously abnormal, the lymph node would not fit easily into the common diagnostic catergories described above.

Diffuse lymphomas with majority neoplastic population

When there is a diffuse lymphoma with majority neoplastic population, the next vital thing is to determine with our panel if these are B or T cells. B-cells would be positive with CD20 and +/- with CD5, but T-cells would be always negative with CD20 and usually but not always positive with CD5 because T-cell lymphomas often lose expression of some pan T-cell markers. Once it is confirmed that the lesion is likely to be a T-cell lymphoma or perhaps may be a B-cell lymphoma which does not express CD20 strongly and uniformly then a referral for assessment with a broader panel will be required.

For CD20 positive diffuse lymphomas with majority neoplastic population, the next step is to assess whether it is of high or low grade by mitotic count/Ki-67 index assessment. High grade lymphomas would have frequent mitoses and a ki-67 index of over 30% diffusely rather that in follicles or growth centres. While low-grade lymphomas would have rare mitoses and a ki-67 index of usually less than 10% outside any growth centres or follicles.

The major diagnostic consideration for a low-grade B-cell lymphoma with a diffuse growth pattern is Small lymphocytic lymphoma/solid phase chronic lymphocytic leukaemia. The tumour cells are small and fairly uniform. It is common and classic to find that there are scattered paler staining foci of larger cells with relatively more abundant cytoplasm. The foci termed pseudofollicles because of their resemblance to germinal centres are populated by para-immunoblasts which have frequent mitoses and form the growth centres for the tumor. These growth centres do not have mantles. The tumour cells in small cell lymphocytic lymphoma are CD20, CD5 and CD23 positive. SLL/CLL may show significant plasmacellular differentiation.

A less common diagnostic possibility for a low-grade diffuse pattern B-cell lymphoma would be Mantle cell lymphoma. The tumor cells here are also small but have irregular nuclei. Growth centres are not present and the tumour cells are positive for CD20 and usually CD5 but not CD23. Confirmation of the diagnosis requires staining with cyclin D1 which is not in our panel hence such cases need referral. As previously mentioned, Mantle cell lymphoma may have a pseudofollicular growth pattern when tumour cells colonise pre-existent follicles. Mantle cell lymphoma can occur with some significant frequency in the distal small and large intestinal mucosa. Marginal zone lymphoma is another uncommon diagnostic consideration for a low grade diffuse B-cell lymphoma in the lymph node, but is much more common in mucosal sites as MALT lymphoma (stomach, salivary glands, thyroid gland) and also in skin as cutaneous marginal zone lymphoma. As mentioned previously, Marginal zone lymphoma may very rarely have a pseudofollicular growth pattern when pre-existent follicles are colonised by tumour cells. Most commonly it forms an expansile sheet-like growth of typically large cells with relatively abundant pale/cleared cytoplasm. The cells occupy the spaces between compressed pre-existent or reactive lymphoid follicles. The tumour cells express CD20, but not CD5 or CD23. It is common for Marginal zone lymphomas to show significant plasmacellular differentiation which would prove to be monoclonal with kappa and lambda light chain immunohistochemistry (not included in our panel). In mucosal sites, the

lymphoid infiltrate of marginal zone lymphoma is often distinguished from an inflammatory infiltrate by the fact that it is expansile and destructive with prominent lympho-epithelial acitivity.

Of the high-grade diffuse growth pattern B-cell lymphomas, there are two which may be diagnosed with our panel if carefully consideration of morphology, clinical presentation and blood/bone marrow findings are done

The first and more common one is the category of diffuse large B-cell lymphoma. This category has a number of variants which will not be detailed here. The diagnostic features that can be used with our panel are the following.

1) That the tumour cells are large (i.e. more than twice the size of a small lymphocyte or the nucleus of a histiocyte) blasts. Their blastic nature can be confirmed by the presence of either a single central prominent nucleolus (immunoblasts) or of multiple (usually three) prominent nucleoli that lie peripherally in contact with the nuclear membrane (centroblasts).
2) That the cells should stain uniformly and strongly with CD20 but not CD5. There are rare occasions when they express CD5, but because we cannot exclude (high grade/blastoid) mantle cell lymphoma with our panel such cases need referral for further assessment.

In diffuse large B-cell lymphoma FDC meshwork is not observed with CD23 though on occasion remnants of residual follicles are seen at the edge of the lymph node or fragments from a disrupted neoplatic meshwork may be identified in parts of the tumour if it is arising from a progression/transformation of a follicular lymphoma.

The second lymphoma that may be diagnosed in this category with our panel is Burkitt's lymphoma. The cells here are usually small round-oval without prominent nucleoli. Mitoses are very frequent as are apoptoses with the presence of numerous tingible body macrophages giving the so called starry sky appearance. This starry sky appearance is typical but not unique as it is merely a reflection of the high turnover of cells due to frequent mitoses/apoptosis with the macrophages there to remove debris. What is unique about Burkitt's is that the Ki-67 index is always very nearly 100%. Indeed it is the practice in some centres to look for the typical c-myc translocation in any diffuse B-cell lymphoma with Ki-67 index of close to 100%. Burkitt's lymphoma cells are CD20 positive, CD10 positive and typically Bcl-2 negative. Any lymphoma that has these features can be safely diagnosed as Burkitt's lymphoma. Otherwise a number of other possibilities arise which would need a wider panel of antibodies to resolve.

Lymphomas in which lymph node architecture is essentially intact.

These final section is included only as a reminder that this can happen as those lymphomas in this category require more than what is our panel for diagnosis. As alluded to earlier examples in this category probably represent early stage of a lymph node involvement. Examples include involvement of sinuses by an anaplastic large cell lymphoma, and of interfollicular regions by a lymphoplasmactyic lymphoma. The important thing is to bear in mind that each lymph node should be examined carefully and if necessary with the help of the antibodies in our panel to ensure that

not only is the architecture intact and cells are where they are supposed to be, but also that no unusual/foreign cells are present.